

What is claimed is:

1. A method for isolating a double-stranded cDNA having a nucleotide sequence of a complete open reading frame which comprises:
- 5
- A) admixing
- (i) an isolated single-stranded cDNA,
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- (ii) a first primer capable of forming a stem-loop structure, comprising
- (a) at the 3' end of the primer, a first random sequence, linked to
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- (b) a second sequence, linked to
- (c) a third sequence which forms a loop structure, linked to
- 20
- (d) a fourth sequence, at the 5' end of the first primer, which is complementary to the second sequence,
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- under hybridization conditions sufficient for annealing the first sequence of the first primer to the sequence at the 3' end of the single-stranded cDNA, and

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(iii) a polymerase;

B) incubating the mixture from step (A) under suitable conditions for DNA synthesis; and

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C) performing a polymerase chain reaction by admixing

(i) an aliquot of the mixture from (B),

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(ii) a second primer which specifically binds to the single-stranded cDNA,

(iii) a third primer which comprises

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(a) a fifth sequence identical to the third sequence of the first primer, linked to

(b) a sixth sequence identical to a portion of the second sequence of the first primer, and

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(iv) a polymerase

25 under conditions suitable for a polymerase chain reaction so as to produce a double-stranded cDNA reaction product, thereby isolating the cDNA having the sequence of the complete open reading frame.

2. The method of claim 1, wherein the single-stranded DNA

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is a 5' portion of a cDNA reverse transcribed from an mRNA.

3. The method of claim 1, wherein the first primer has the sequence

3'-

NNNNNNNNNNNNNCAGAGCTCAAATTTGTGATCAGCTGGTCTTTCACAAATTTGAGCTCTG-5' (D-SLAP).

4. The method of claim 1, wherein the first primer has the sequence

3'NNNNNNNNNNNGGGAGAGCTCACAGCTGAAGCAGCTGACTAGCACCTAGTGTAGAATACATCTTGAGCTAT-5' (D-CLAP1).

5. The method of claim 1, wherein the first primer has the sequence

3'NNNNNNNNNNNNNAGAGCTCACAGCTGAAGCAGCTGACTAGCACCTAGTGTAGAATACATCTTGAGCTAT (D-CLAP2)

6. The method of claim 1, wherein the first primer comprises an inosine nucleotide.

7. The method of claim 1, wherein the loop structure is a simple loop structure, or a cloverleaf loop structure.

8. A method for generating a cDNA library which comprises:

A) admixing

(i) a population of single-stranded cDNA molecules which were reverse transcribed with an oligo-dT sequence linked to a defined nucleotide sequence,

5 (ii) a first primer capable of forming a stem-loop structure, comprising

(a) at the 3' end of the primer, a first random sequence linked to

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(b) a second sequence, linked to

(c) a third sequence which forms a loop structure, linked to

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(d) a fourth sequence, at the 5' end of the first primer, which is complementary to the second sequence,

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under hybridization conditions sufficient for annealing the first sequence of the first primer to the sequence at the 3' end of the single-stranded cDNA, and

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(iii) a polymerase;

B) incubating the mixture from step (A) under suitable conditions for DNA synthesis by the polymerase; and

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- (i) an aliquot of the mixture from (B),

(iii) a third primer which comprises

(b) ~~a sixth sequence identical to a portion of the second sequence of the first primer, and~~

9. The method of claim 8, wherein the single-stranded DNA
25 is a cDNA reverse transcribed from an mRNA.

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AGCTCTG-5' (D-SLAP).

11. The method of claim 8, wherein the first primer has the sequence

5 3'NNNNNNNNNNNGGGAGAGCTCACAGCTGAAGCAGCTGACTAGCACCTAGTGTAGAAT
ACATCTTGAGCTAT-5' (D-CLAP1).

12. The method of claim 8, wherein the first primer has the sequence

10 3'NNNNNNNNNNNNNAGAGCTCACAGCTGAAGCAGCTGACTAGCACCTAGTGT
AGAATACATCTTGAGCTAT (D-CLAP2).

13. The method of claim 8, wherein the first primer comprises an inosine nucleotide.

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14. The method of claim 8, wherein the loop structure is a simple loop structure, or a cloverleaf loop structure.

15. A kit for the generation of a complete open reading
20 frame double-stranded cDNA of interest which comprises:

(i) a first primer capable of forming a stem-loop structure, comprising

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(a) at the 3' end of the primer, a first random sequence linked to

(b) a second sequence, linked to

(c) a third sequence which forms a loop

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(d) a fourth sequence, at the 5' end of the first primer, which is complementary to the second sequence, and

(a) a fifth sequence identical to the third sequence of the first primer, linked to

16. A method for isolating a double-stranded cDNA having a nucleotide sequence of a complete open reading frame which comprises:

(i) a biological sample containing mRNA,

(a) a poly-T sequence at the 3' end of the primer linked to

(b) a first random sequence linked to

(c) a second sequence ~~which~~ forms a loop

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structure linked to

5 (d) a third sequence at the 5' end of the primer
which is complementary to the first
sequence, and

(iii) a reverse transcriptase,

10 under hybridization conditions sufficient for annealing
the primer to the mRNA poly-A sequence;

(b) incubating the mixture from step (a) under suitable
conditions for reverse transcription;

15 (c) performing a polymerase chain reaction with an aliquot
of the mixture from step (b) using one gene-specific
primer which is pre-defined and one primer which has a
sequence identical to at least a portion of the primer
sequence of element (ii), thereby isolating the cDNA
20 having the sequence of the complete open reading frame.

25 17. The method of claim 16, wherein the primer has the
s e q u e n c e 3 ' -
TTTTTTTTTTTCAGAGCTCAATTGTGATCAGCTGGTCTTTCACAAATTG
AGCTCTG-5' (T-SLAP).

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